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CLAIMS

1. A method of producing a hybrid DNA molecule having a sense strand and an anti-sense strand and in which, reading in the 5' to 3' direction, the sense strand has the sequences x_1, x_2.....x_n, where n is greater than or equal to 3, the method comprising the steps of

(1) providing in a single reaction mixture

(a) the sequences x_1, x_2.....x_n and their complementary sequences x_1', x_2'.....x_n', to be assembled into the hybrid molecule,

(b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers hybridising to the 3' ends of any two sequences (x_i, $x'_{(i+1)}$), where i is 1 to (n-1), have specifically complementary linker sequences

(2) effecting a first stage PCR reaction in which those primers provided with linker sequences are present in limiting concentrations, and

(3) effecting a second stage PCR reaction using a single pair of primers one of which provides the 5'-end of the sense strand and other of which provides the 3'-end of the anti-sense strand of the required hybrid molecule

whereby said hybrid molecule is generated.

2. A method as claimed in claim 1 wherein the polymerising enzyme adds a 3' adenosine overhang to an extended strand and those primers incorporating linker

sequences have their priming sequences connected to their respective linker sequences via an adenine residue.

3. A method as claimed in claim 2 wherein the polymerising enzyme is *Taq*.

4. A method as claimed in any one of claims 1 to 3 wherein the annealing temperature (T_m) of the linker sequences is greater than that of the priming sequences to the x and x' sequences.

5. A method as claimed in claim 4 wherein the annealing temperature of the linker sequences is 2 to 5°C greater than that of the priming sequences to the x and x' sequences.

6. A method as claimed in any one of claims 1 to 5 wherein the linker sequences do not have intrinsic secondary structure.

7. A method as claimed in any one of claims 1 to 6 wherein between the first and second stage PCR reactions the reaction mixture is frozen to deactivate residual PCR activity.

8. A method as claimed in any one of claims 1 to 6 wherein between the first and second stage PCR reactions the reaction mixture is treated with an exonuclease I to digest single stranded molecules.

9. A method as claimed in any one of claims 1 to 8 wherein each of the first and second stage PCR reactions utilise a thermally activated polymerase.

10. A method of mutation analysis wherein the analysis is effected on a DNA hybrid molecule produced in accordance with the method of any one of claims 1 to 9.

11. A set of primers incorporating the following sequences.

5'tcatattagccgctgcattgcc-a-3'

5'ggcaatgcagcggctaatatga-a- 3'

5'agccactacccaaaactcctgt-a-3'

5'acaggagtttgggtagtggct-a-3'

5'tgtctcactgaacctgcctacct--a-3'

5'aggtaggcaggttcagtgagaca-a-3'

5'cctcat taccggctgtcagactg-a-3'

5'cagtctgacagccggtaatgagg-a-3'

12. A method of producing a hybrid DNA molecule having a sense strand and an anti-sense strand and in which, reading in the 5' to 3' direction, the sense strand has the sequences x_1, x_2.....x_n, where n is greater than or equal to 3, the method comprising the steps of

(i) providing in a single reaction mixture

(a) the sequences x_1, x_2.....x_n and their complementary sequences x_1', x_2'.....x_n', to be assembled into the hybrid molecule,

(b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers for the 3' ends of any two sequences (x_i, $x'_{(i+1)}$), where i is 1 to (n-1), have specifically complementary linker sequences connected to their respective priming sequences via an adenine residue, and

(g) effecting a PCR reaction using a polymerase which adds a 3' adenine overhang to the end of an extended strand